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Indirect geometry measurement method based on confocal microscopy and fluorescent microparticles

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ABSTRACT

The precise production of micro- or micro-structured components of increasingly different materials requires ever more precise and flexibly adjustable geometry measurement methods. Today's optical metrology offers various innovative approaches for this purpose. A major shortcoming is, however, that not all surfaces and structures to be measured are optically cooperative and return too little light or information to the measurement system for the signal analysis. Therefore, indirect optical geometry measurements are introduced as a new approach: Instead of directly detecting the outer boundary layer of the measuring object, the shape of the object's imprint in the surrounding medium is examined. For this purpose, the surrounding medium is enriched with fluorescent substances and a confocal microscope scans the space surrounding the measured object. The spanned area above which the fluorescence signal disappears is then determined as the boundary layer between the measurement object and the surrounding medium. As a result, the object geometry is obtained completely independently of the optical response behavior of the object. While first realizations studied measurements in a liquid environment, this work demonstrates for the first time the feasibility of indirect optical geometry measurements in air environments with the aid of fluorescent microparticles. In order to maximize the measurement accuracy, different model-based signal evaluation approaches for determining the interface geometry from the fluorescence signals are investigated and compared, taking both cases (liquid and air environment) into account. Finally, indirect optical measurements are performed on a step geometry, reconstructing the height profiles using the theoretically derived model function.

Keywords: indirect optical geometry measurement, confocal fluorescence microscopy, fluorescent microparticles

1. INTRODUCTION

There are a large number of high-precision optical geometry measurement methods, especially for manufacturing technology. In order to categorize the number and types alone, Hocken et al [1], as well as Whitehouse [2], divide the systems into 14 classes. The methods range from microscope systems [3] and optical profilers [4] to speckle measurement methods [5] and holographic systems [6], with the measurement range extending from the meter range of fringe projection to the nanometer range of near-field optical microscopy.

Despite this great variety, however, all optical methods require a sufficiently large proportion of light energy that is scattered or reflected from the surface and can be detected with a photodetector or camera. For this reason, the scattering behavior of the surface to be measured in combination with the chosen measurement approach currently determines the measurement success. In fact, special surface-specific measurement solutions have been developed for each type of surface. For example, deflectometry [7] works on highly reflective surfaces, but fringe projection [8] is more preferable on matt surfaces.

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The revolutionary measurement approach presented here leads to a paradigm shift in optical geometry measurement, as it works completely detached from the optical properties of the object to be measured. The basic idea here is not to measure the specimen itself, but in an inverse process only its imprint in the surrounding medium.

The principle of this indirect geometry measurement is based on enriching the environment with fluorescence particles. A confocal fluorescence microscope then scans the surrounding space with its confocal volume and measures the intensity of the fluorescence. Low-intensity points where the fluorescence disappears are located inside the object to be measured and define the imprint of the specimen or the boundary layer of the surface geometry.

The transition from the fluorescence of the environment to the darkness in which the confocal volume is located in the sample body is of course not discrete. The intensity curve depends on the density and speed of the fluorescence particles as well as on the confocal volume of the fluorescence microscope. Finding and defining the actual surface position is hence a non-trivial problem. Therefore, in order to exploit the resolution potential of the confocal fluorescence microscope, a model-based measurement is required, which determines the point of the surface in the intensity curve.

The scientific aim of this contribution is to develop the theoretical model for the intensity profile in confocal fluorescence microscopy using a fluorescent aerosol. On the basis of the model, the first indirect geometry measurements and a quantitative evaluation will then be carried out.

2. THEORY AND METHODS

2.1 Indirect optical geometry measurement method and confocal fluorescence microscopy

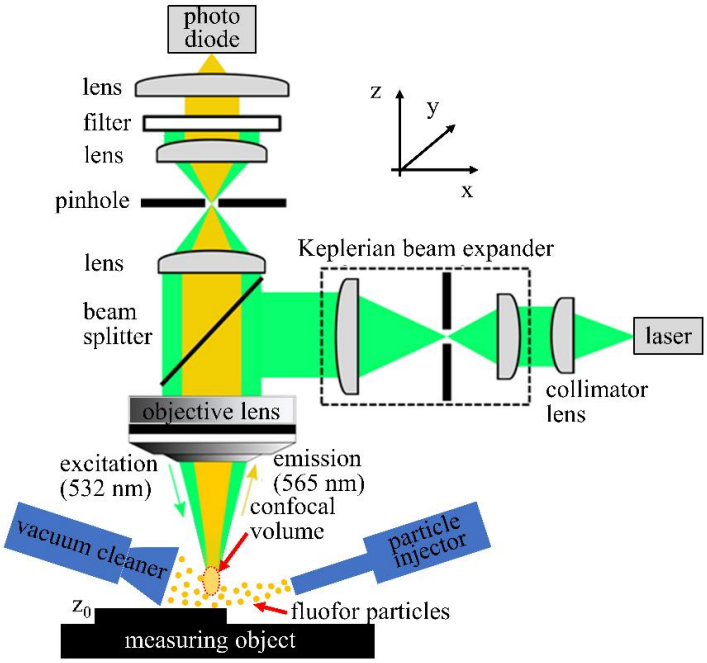

Figure 1: Principle of indirect optical geometry measurement using a confocal fluorescence microscope.

Figure 1a shows the basic measurement setup of a confocal fluorescence microscope. The excitation laser with a wavelength of 532 nm is coupled into the microscope via a Keplerian beam expander and focused with the objective lens in the confocal volume. The pinhole and the bandpass filter in the beam path of the microscope ensure that only fluorescence light from the confocal volume reaches the photodetector.
At each individual point of the *xy* surface, the fluorescence microscope now scans the spatial volume around the measurement object with its confocal volume along the optical *z*-axis. Above the surface of the measurement object, a clear fluorescence signal can be expected, corresponding to the fluorescence particle density. The more the confocal volume penetrates the measurement surface, the weaker the signal becomes until it finally disappears completely. With the help of model-based signal analysis, the true value z_0 of the surface position must be found.

This completely new approach to optical geometry measurement was first described in 2016 by Takaya et al. for the in-situ measurement of cutting tools in a manufacturing process [9, 10]. Here, the fact that the tool is basically wetted with cooling lubricant in the process is exploited. Takaya enriches the cooling lubricant with fluorescent dye and can thus determine the geometry and wear of the tool. For the case of a very thin liquid film, he approximates the intensity distribution in the z-direction along the optical axis of the fluorescence microscope by a Gaussian curve with the maximum at z_0 (see also Figure 2 in Section 2.2).
Mikulewitsch et al. [11, 12] in 2020 goes one step further and does not measure the surface boundary layer wetted with fluorescent dye, but instead pursues the concept of Indirect Geometry Measurement (InOGeM) for the first time by not measuring the object itself, but its imprint in the surrounding medium. Mikulewitsch et al. measures the fluorescence response of the process liquid enriched with a fluorescent dye in the wet-chemical laser ablation process, and thus the volume of liquid surrounding the measurement geometry. For the model-based evaluation of the surface geometry, they deal intensively with the subsequent theory of confocal fluorescence microscopy.

2.2 Model-based geometry measurement

The intensity measured by the detector over z is shown in Figure 2 for different fluorophore distributions. The intensity signal is significantly influenced by two factors. Firstly, by the spatial excitation energy $I(z)$ of the fluorescent dye in the confocal volume, and secondly by the fluorescence response of the surrounding medium $\eta(z)$. While the medium and the measured object are in a spatially static state, the confocal volume is moved in the z-direction during the measurement. The resulting detector intensity thus results from the z-dependent convolution

$$I_F(z) = \eta(z) * I(z). \tag{1}$$

The convolution integral can be written as:

$$I_F(z) = \int_{-\infty}^{\infty} \eta(\tilde{z}) I(z - \tilde{z})\, d\tilde{z} \tag{2}$$

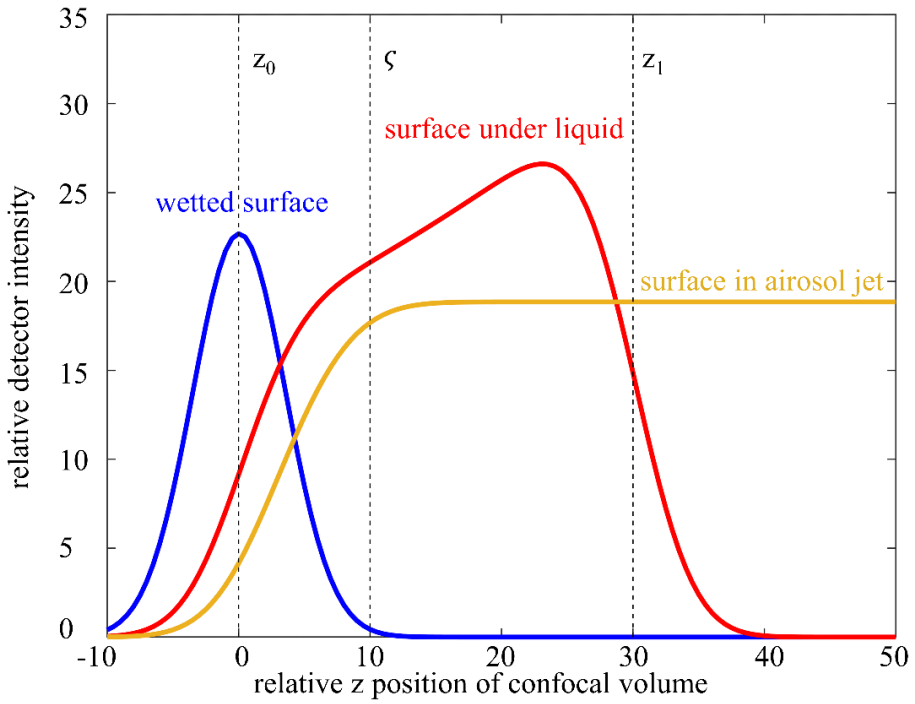

Figure 2: Model functions for the z-dependent intensity distribution in confocal fluorescence microscopy for different approaches of a fluorophore distribution.

For the simple example of an infinitely thin wetted surface considered by Takaya, a delta distribution function $\delta(\tilde{z} - z_0)$ can be assumed as the fluorescence response of the medium $\eta(z)$, where z_0 is the position of the surface. For the laser, a constant, homogeneous power with an amplitude of 1 is assumed in the following, so that the excitation energy is determined only by the Gaussian spatial distribution of the confocal volume $e^{-\frac{2}{\omega_0^2}\left(\vec{r}^2 - \frac{z^2}{\kappa^2}\right)}$ [13]. The width of this distribution is expressed by the angular frequency ω_0 in the *xy*- or $\vec{r} = \binom{x}{y}$-direction and by $\kappa \cdot \omega_0$ in the *z*-direction, and scales with a constant factor κ depending on the detection aperture of the confocal system. The convolution integral (2) thus results in:

$$I_F(z) = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} \delta(\tilde{z} - z_0) e^{-\frac{2}{\omega_0^2}\left(\vec{r}^2 - \frac{(z-\tilde{z})^2}{\kappa^2}\right)} d\vec{r}\, d\tilde{z} \tag{3}$$

It is known from signal theory that the convolution of a Gaussian function with delta distribution results in the same Gaussian function again. For the intensity profile in the context of a *z*-scan, this results in the blue curve shown in Figure 2. The position of the surface (z_0) is at the maximum of the intensity distribution.

In their problem - of the measured object within a liquid – Mikulewitsch et al. set a rectangular function (1 for $z_0 < z < z_1$ and 0 otherwise) for the response function of the medium η and solves the convolution integral (2) analytically [11]:

$$I_F(z) = \Psi(I_0, \omega_0\kappa, \varepsilon) e^{\varepsilon(z+z_0-z_1)} \left(erf\left(\frac{z}{2\xi} + \varepsilon\xi\right) - erf\left(\frac{z-z_1}{2\xi} + \varepsilon\xi\right)\right), \quad \text{with} \quad \xi = \frac{\kappa\omega_0}{2\sqrt{2}}\,. \tag{4}$$

This curve is shown as a red line in Figure 2. In addition to the immersion of the confocal volume into the surface z_0, it can also be seen what happens when the confocal volume is no longer in the fluorescent medium, i.e. above z_1.

The question regarding the model function for the flow of individual fluorophore particles shown in Figure 1 and the response function of the medium to be applied here will be clarified experimentally and analytically in the following.

3. RESULTS

3.1 Measurement setup and experimental realization

The practical realization of the measurement setup described in Figure 1 can be seen in Figure 3a.

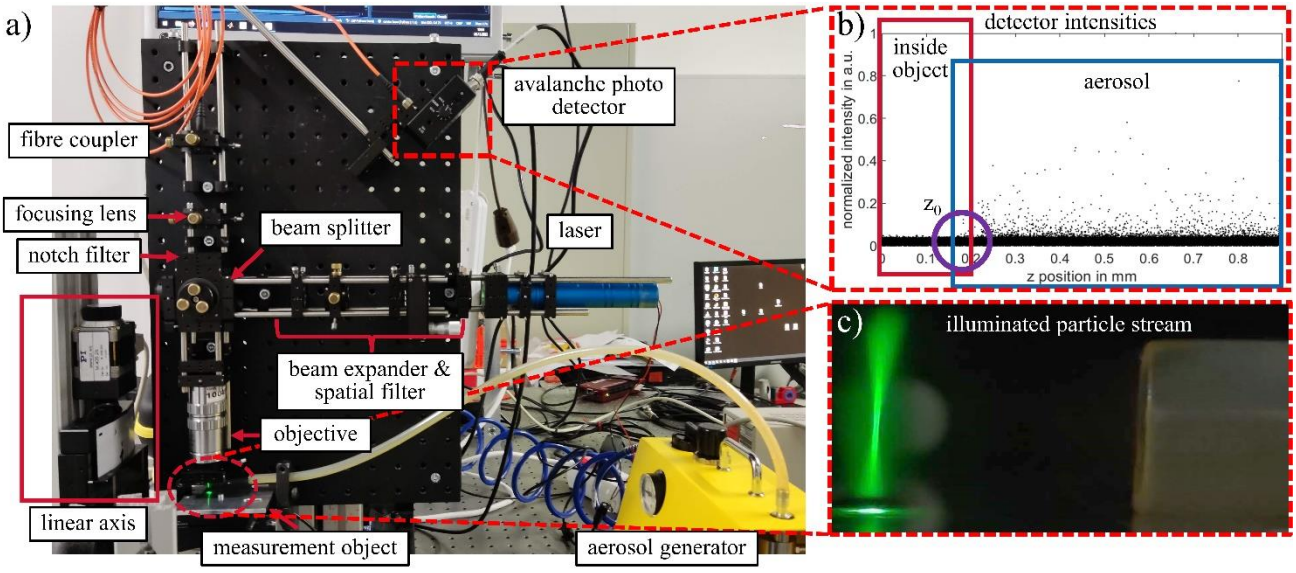

Figure 3: Realization of the confocal fluorescence microscope: a) measurement setup with particle generator, b) exemplary intensity measurement for a scan along the optical z-axis and c) enlarged section of the confocal volume of the fluorescence microscope with the aerosol feed.

The green continuous wave excitation laser from *Edmund optics* (532 nm, 50 mW) is coupled via a beam splitter into the confocal volume of the infinity-corrected Optem Plan Apo 20X lens from *Qioptiq*. The fluorescence response signal is transmitted via the notch filter (532+/-17 nm) and the multimode fiber to the avalanche photo detector APD440A2 from *Thorlabs*. The step size of the linear units from *PI* is 0.05 mm in the *z*-direction and 0.1 mm in the *xy*-direction.

The fluorophore aerosol has an average particle size of 1 µm and is generated using an aerosol generator from the company *topas*. The fluorescence dye used, pyrromethene 567, emits at 567 nm and is dissolved in DEHS.

Figure 3c shows the particle flow illuminated by the excitation laser, with the maximum in the 10 µm confocal volume of the fluorescence microscope, at an illumination time in the ms range. The particle velocity is approx. 1 m/s. The dwell time of a particle in the confocal volume is therefore 10 µs, which corresponds exactly to the integration time of the avalanche diode. Since the particle density is very low, particles are only detected relatively rarely, as can be seen in Figure 3b. The probability of a measurement is only 5 per mil and correlates directly with the speed of the particles.

An analysis of the flow velocities of the fluorescence particles is carried out in the following section on the basis of Particle Image Velocity (PIV).

3.2 PIV measurements of the flow velocities of the fluorescence particles

To determine the flow velocity distribution of the fluorescence particles, a commercial PIV system from *Dantec Dynamics* is used. Measurements are taken directly in the measurement setup shown in Figure 3a, on the step object in Figure 5b. The associated flow profile is shown in Figure 4.

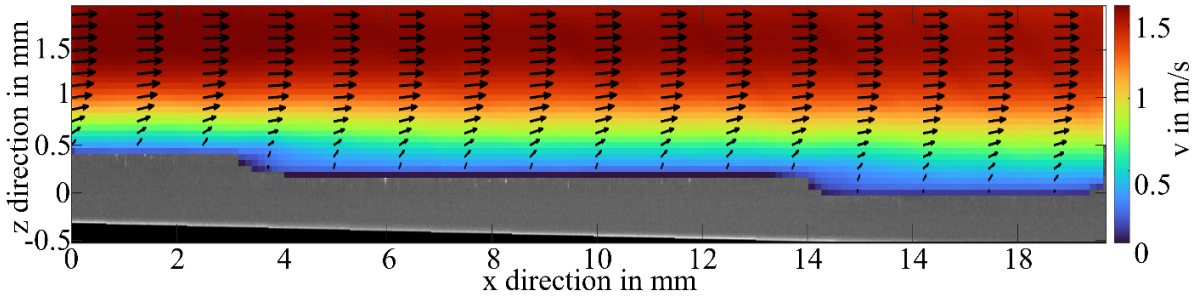

Figure 4: PIV measurements of the velocity vector field in the free jet of the aerosol particle generator above the step profile from Figure 5b.

According to the fluid-dynamic boundary layer theory, the velocity profile $v(z)$ of a medium flowing laminarly against an infinitely large flat plate results in a first approximation from the quadratic equation

$$v(z) \approx v_0 \left[1 - \left(1 - \frac{z}{\varsigma}\right)^2\right]. \tag{5}$$

Here v_0 is the velocity of the free jet and ς the thickness of the velocity boundary layer.

The PIV measurements of the flow field (Figure 4) confirm the simplified assumption of a quadratic relationship, so that equation (5) can be used to model the intensity measurements.

3.3 Particle-based model of intensity distribution

As indicated in section 3.1 (Figure 3b), the z-dependent intensity measurement results from a purely statistical process. The higher the particle velocity, the greater the probability - because of the low particle density - that a fluorescent particle will pass the detector and contribute to the measurement. The fluorescence response of the surrounding medium $\eta(z)$ from equation (1) is therefore directly proportional to the velocity distribution from equation (5).

If the intensity distribution of the confocal volume $e^{-\frac{2}{\omega_0^2}\left(\vec{r}^2-\frac{z^2}{\kappa^2}\right)}$ is again used for the excitation energy *I(z)* of the fluorescent dye, equation (1) can be directly rewritten as:

$$I_F(z) = v_0\left[1-\left(1-\frac{z}{\varsigma}\right)^2\right] * e^{-\frac{2}{\omega_0^2}\left(\vec{r}^2-\frac{z^2}{\kappa^2}\right)} \tag{6}$$

The corresponding convolution integral is as follows:

$$I_F(z) = \iint_{-\infty}^{\infty} v_0\left[1-\left(1-\frac{\tilde{z}-z_0}{\varsigma}\right)^2\right] e^{-\frac{2}{\omega_0^2}\left(\vec{r}^2-\frac{(z-\tilde{z})^2}{\kappa^2}\right)} d\vec{r}\,d\tilde{z} \tag{7}$$

Since the velocity distribution from equation (5) is not defined at infinity, equation (7) is not yet mathematically correct and must be extended to include Heaviside functions, which ensure that the particle velocity is zero in the range smaller than z_0 and increases slowly up to v_0 in the remaining range.

$$I_F(z) = \iint_{-\infty}^{\infty} v_0\left\{\mathcal{H}(-\tilde{z}+\varsigma+z_0)\mathcal{H}(\tilde{z}-z_0)\left[1-\left(1-\frac{\tilde{z}-z_0}{\varsigma}\right)^2\right]+\mathcal{H}(\tilde{z}-\varsigma-z_0)\right\} e^{-\frac{2}{\omega_0^2}\left(\vec{r}^2-\frac{(z-\tilde{z})^2}{\kappa^2}\right)} d\vec{r}\,d\tilde{z} \tag{8}$$

This integral can be solved analytically using *Matlab* and is shown as a yellow curve in Figure 2.

While the position z_0 is located in the maximum of the Gaussian curve for the thinly wetted surface, both Takaya et al. and Mikulewitsch et al. demonstrate for extended liquid layers that the point of the surface is located here in the inflection point of the error function (4) used (red curve). In the case of particle flow (yellow curve), this symmetry is broken due to the applied inhomogeneous particle velocity, and the point z_0 of the surface is reached significantly earlier in the intensity profile.

3.4 Results of Indirect Optical Geometry Measurement

In order to carry out the statistical evaluation of the measurement data in Figure 3b and to be able to determine the position z_0, the detector noise must first be minimized. For this purpose, a Grubbs outlier test is carried out in the measurement data. Only the measured values that are recognized as outliers are counted, all others are set to zero. After a slight low-pass filtering over 400 measured values or 4 ms, the blue data points in Figure 5a result. The convolution integral for the intensity function of the confocal fluorescence microscope using a particle stream (equation (8)) is fitted into these data points using a least square algorithm and the free parameters z_0, ς and v_0 are determined. The fit function is shown in red in Figure 5a, the value for the calculated free parameter z_0 is shown as grey dashed lines and the standard deviation S for this value is also given. Measured were the *z*-scans at the positions x = 6 mm, 13.5 mm and 20 mm.

The calculated position z_0 of the surface varies over the course of the curve and is not always at the inflection point. Rather, it is clearly shifted to the left towards the zero line and thus cannot be determined by simple gradient analysis as in [9]. How close the point of the first measurable statistical outliers is to the real surface, and how high the measurement resolution is, is largely determined by the confocal volume of the fluorescence microscope.

To show the independence from the optical properties of the surface, the step geometry as a measurement object was colored black with soot particles before the measurement (Figure 5b) and thus optically passivated. The line measurement along the x-axis of the step geometry for all 200 individual intensity scans in the z-direction is shown in Figure 5c. The deviation between the WLI reference measurement and the InOGeM measurement, for the approximately 200 µm high step (AB), is 1.4 µm. The deviation of 1.7 µm is somewhat higher for the step (BC) due to the small number of measurement data points, but with a standard deviation of the InOGeM measurement of approx. 20 µm it is clearly within the measurement uncertainty.

These first measurements show that, with the help of a statistical, model-based evaluation, an indirect optical geometry measurement is possible within an aerosol atmosphere of fluorescent microparticles surrounding the measurement object.

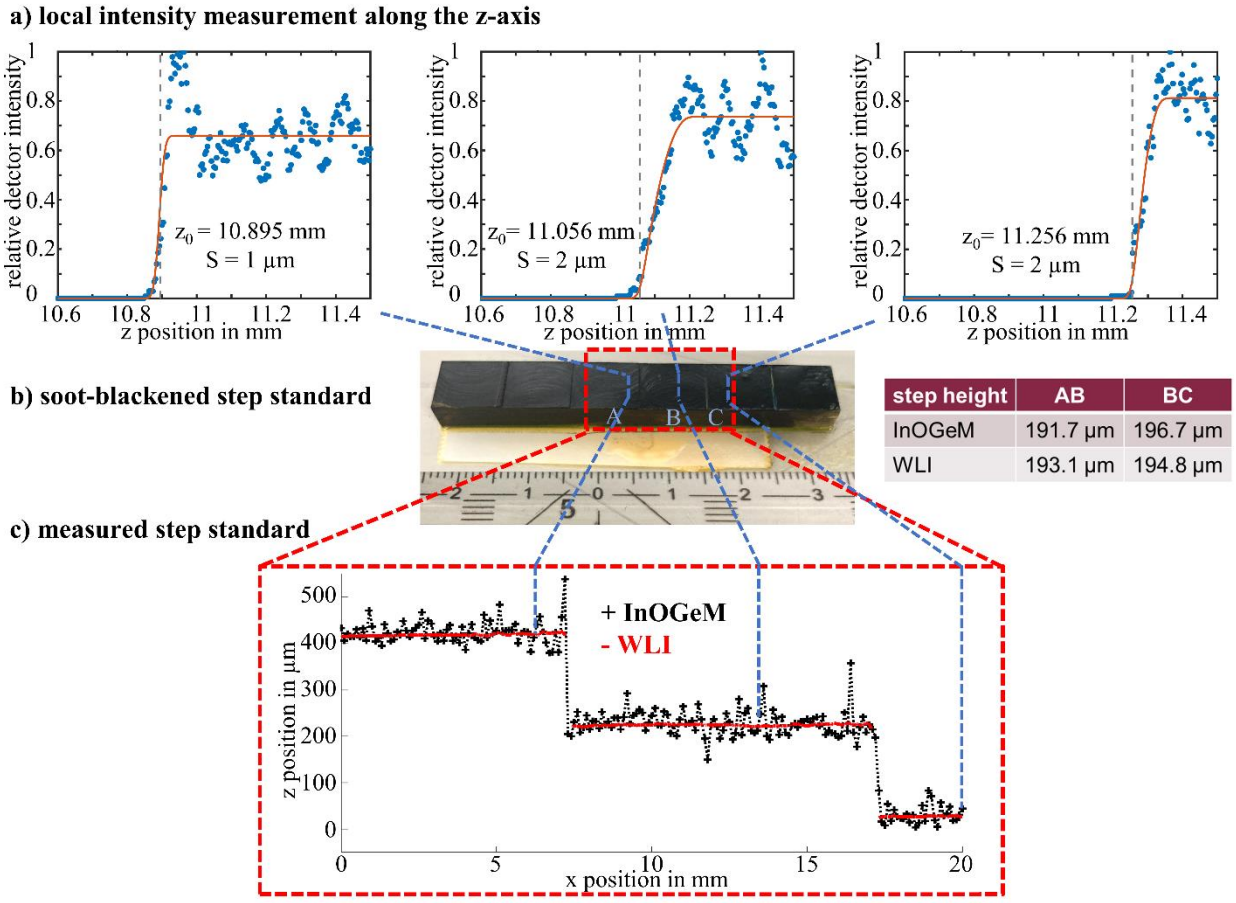

Figure 5: Indirect optical geometry measurements based on confocal fluorescence microscopy. a) local intensity distributions measured along the optical axis, b) soot-blackened step geometry with steps approx. 200 µm high and c) height profile of the step reconstructed using the model function, compared with a white light interferometer (WLI).

4. CONCLUSION AND OUTLOOK

In the first approaches to indirect optical geometry measurement mentioned so far, liquids are used, whereby the model-based calculation of the geometry is mainly determined by the course of the surface boundary layer. The completely new approach presented here uses an inflowing aerosol of fluorophore particles as measuring probes. The model-based evaluation used to calculate the surface geometry considers the medium surrounding the measuring body, including the velocity distribution of the aerosol, which influences the fluorescence intensity. A quadratic course is postulated for the velocity distribution according to flow theory and verified by means of a PIV measurement. The model for the intensity distribution measured with the confocal fluorescence microscope is determined on the basis of the properties of the medium and the confocal volume using the convolution integral. Due to the low fluorescence particle density, the measurement data entering the model are recorded via a statistical evaluation. The real geometry is determined by fitting the model to the data using a least square algorithm and assigning the free parameter z_0 to the point of the surface.

Overshooting of the system at steep edges often occurs with optical methods. Particularly at the transition of the BC stage (Figure 5c), it can be seen that this problem does not occur in principle with the InOGeM system, since the method is decoupled from the optical properties of the surface. Despite this universal approach, the resolving power, especially with gases as the fluorescent medium, is limited only by the confocal volume of the fluorescence microscope. Already in the results shown here with aerosols, measurements with deviations from the reference method of 1.4 µm, with standard deviations of less than 20 µm and without optical overshooting at the edges, could be achieved at 200 µm high optically uncooperative stages.

With regard to the measurement uncertainty of the method used, there is room for optimization with a current confocal volume in the single-digit µm range. Therefore, to improve the resolution, the next step is to work out the technical details of the particle feed, the homogenization of the particle size and the particle density at the edges. In the long term, in order to overcome the particle problem completely, research is to be carried out on measurement in fluorescent gases such as CO_2. Particularly in the production of high-precision micro components or micro structured surfaces, depending on the process, a variety of specially adapted complex methods for in-process measurement and quality inspection have been used up to now. Confocal fluorescence microscopy offers the potential to reduce this multitude of required procedures also for the application on optically uncooperative materials and thus to revolutionize optical geometry measurement as an universally applicable measurement method in the future.

Author Contributions:

A. Tausendfreund and A. Fischer conceptualized the experiments. A. Tausendfreund further developed the model approach, B. Espenhahn carried out the PIV measurements and realized the measurement setup and G. Behrends was responsible for the measurements with the fluorescence microscope. A. Fischer was responsible for supervision and finally reviewed and edited the manuscript.

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